A COMPARATIVE ANALYSIS OF THE BIOCHEMICAL AND PHYSIOLOGICAL ROLE OF THE MATERNAL AND AMNIOTIC ENVIRONS IN NORMAL AND DIMETHYL-SULFOXIDE-INDUCED CHANGES IN MACRO- AND MICROMOLECULES OF THE LONG-EVANS RAT DURING FETALOGENESIS

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ABSTRACT

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A Comparative Analysis of the Biochemical and Physiological Role of the Maternal and Amniotic Environs in Normal and Dimethylsulfoxide-Induced Changes in Macro- and Micromolecules of the Long-Evans Rat During Fetalogenesis

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A sequential study was carried out on maternal serum, fetal serum, and amniotic fluid from 13-1/2 - 20-1/2 days of gestation. This study was conducted to determine the regulatory role macromolecules such as serum-like proteins in amniotic fluids played in homeostasis during fetalogenesis. The total protein concentration in maternal serum fluctuates throughout pregnancy. From 13-1/2 to 18-1/2 days of gestation the total protein concentration exceeds that of non-pregnant rat serum. As gestation advances total protein of amniotic fluid and fetal serum also increases. The total protein of the amniotic fluid increases from 0.095 g/100 ml on 13-1/2 days of gestation to a mean high of 0.25 g/100 ml on 20-1/2 days of gestation. The total protein of fetal serum in 13-1/2-day fetuses showed a mean of 0.403 g/100 ml. The mean total protein in fetal rat serum increases to 2.4 g/100 ml by day 20-1/2.

Albumin fractions in fetal serum and amniotic fluid represent

more than 60% of the total protein. Densitometric scans show that as many as 6 bands can be identified in the amniotic fluid and fetal serum using Cyanogum-41 (polyacrylamide) gel electrophoresis. Large amounts of prealbumin are present during most of the gestational periods in the amniotic fluid and fetal serum. The amniotic fluid and fetal serum are very similar. Sodium and potassium concentrations in the amniotic fluid and maternal serum are very similar for the same gestation days. Sodium and potassium concentrations in the fetal serum are greater than that of maternal serum and amniotic fluid for 13-1/2 - 20-1/2 days of gestation.

When maternal rats are subjected to dimethylsulfoxide (DMSO) treatment, this apparent sequential change in blood serum and amniotic fluid protein profiles is abolished. Observable electrolyte changes in these compartmentalized fluids are also evident.

Gravid female rats injected on 14-1/2 days with 75% DMSO (0.25 ml) show a decrease in the total protein of the maternal serum on 15-1/2 days of gestation. There is no apparent change in total protein concentration on 19-1/2 days of gestation in the maternal serum. There was no significant change in the total protein concentration of fetal serum and amniotic fluid from DMSO-treated gravid rats. DMSO caused a sharp decrease in the sodium levels in 15-1/2 and 19-1/2 day fetal serum and amniotic fluid. The effect on potassium levels was negligible. Thus, dimethylsulfoxide can induce electrolyte disturbances and changes in the concentration of proteins fractions in amniotic fluid and fetal serum most drastically at 24 hrs post-treatment.

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CHAPTER I

INTRODUCTION

Amniotic fluid is a transient liquid of uncertain status. It begins to appear with the formation of the extra-embryonic membranes, reaches a maximum in most species somewhat prior to the end of gestation, and is dissipated at parturition. Obvious functions of amniotic fluid are to provide thermal insulation, to cushion the embryo from shock and from abrasion by the uterine wall, and to provide a spacious moist environment in which the embryo can develop and move about in relative weightlessness. More subtle functions of amniotic fluid such as nourishment and regulation of fetal hydration or hydration remain an enigma (Harvey, 1972). Because of the amnion's limited duration, regulation of change in its fluid volume and contents remains unaccounted for by its generally related function.

Most of the chemical constituents of maternal and fetal plasma are probably demonstrable in the amniotic fluid, and the study of its relative concentrations plays an important part in understanding the physiology of gestation. The postulation above is tangibly fortified by studies of amniotic fluid which have permitted the prenatal prediction of the fetal condition in the various diseases which complicate pregnancies and induce fetal anomalies.

Studies by Brzezinski et al. (1961) in human beings showed a similarity in the electrophoretic pattern of amniotic fluid and fetal serum and also showed that at term, larger differences existed in the

relative concentrations of protein components when maternal serum and amniotic fluid were compared.

The specific nature of the proteins found in the amniotic fluid should provide information as to their source. Unfortunately, the various studies on this problem are not in total agreement. McKay et al. (1958) suggested that amniotic fluid during early human pregnancy has the general protein structure of maternal interstitial fluids which are, presumably, dilute dialysates of maternal serum. In support of this, Abbas and Tovey (1960) demonstrated the similarity of electrophoretic patterns of amniotic fluid proteins and those of maternal serum which had been dialyzed across fetal membranes.

In medicine, DMSO has been shown to cross the dermal barrier and in high concentrations. It accomplished this with little or no permanent tissue damage (Kiligman, 1965). Lysozyme, as an exemplary protein, has been shown to assume a configuration, when suspended in DMSO, with greater flexibility than the native protein without change in its molecular weight (Hamaguchi, 1964). It was observed in these studies that the major configurational changes occurred with DMSO concentrations between 60 to 70%. In blood serum proteins, it was suggested that because of its comparatively small size DMSO is able to penetrate regions on the albumin subunit interfaces more readily than ethylene glycol, polyethylene glycol, glycerol, and sucrose (Herskovits and Laskowshi, 1962).

The objectives of this research were: (a) to determine the sequential changes and comparative differences that occur in protein composition of maternal serum, amniotic fluid and fetal serum during the

gestational periods of 13-1/2 - 20-1/2 days; (b) to advance the knowledge of the sequential and varied changes in the osmotic pressure in the amniotic fluid occurring during rat fetalogenesis (McMillan, 1971); (c) to determine the physiological role of macromolecular changes in amniotic fluid by experimentally inducing fluid and electrolyte imbalances using DMSO; and (d) to provide data which will elucidate the origin of the amniotic fluid and its physiological role in maintaining a homeostatic environment during fetalogenesis.

CHAPTER II

REVIEW OF LITERATURE

Origin and Composition of Maternal Serum and Amniotic Fluid

Vosburgh et al. (1948) showed what appears to be important evidence that water in the amniotic fluid is replaced from maternal plasma every 2.9 hrs. It was assumed that an equal amount of water is transferred in the opposite direction, from the amniotic fluid to mother, thus producing a continuous exchange of water between these two compartments.

Rosa (1951) found evidence of secretory potentialities in amniotic epithelium. Hutchinson et al. (1955) utilized transfer rates of sodium, potassium, and hydrogen isotope tracers to find that amniotic fluid was not a "transudate or dialysate of maternal plasma", but that each element exchanged at its own rate and was in dynamic equilibrium with the maternal system.

According to McKay et al. (1958), the general protein composition of fluids from the embryonic side of the trophoblast resembled the protein composition of maternal serum. In general, there appears to be a lower relative concentration of all globulins in chorionic fluid and amniotic fluid than in maternal serum. The differences in relative ratios of beta and gamma globulin were too slight to be of any significance. These investigators suggested the possibility that proteins from the embryonic fluids are derived from maternal serum by transfer across the trophoblast. The fluids have a slightly higher albumin and globulin ratio than serum and in this respect resemble other interstitial fluids of the adult such as lymph and ascitic fluids, which are

presumably dilute dialysates of serum.

From the similarities in protein patterns, Brzezinski (1961) considered the possibility of fetal circulation supplies proteins to the amniotic fluid. Absence of large protein molecules in amniotic fluid, such as lipoproteins and fribrinogen, and a reduction of gamma globulins, clearly indicated that proteins of the pools serving as sources of amniotic fluid undergo sequestration, perhaps by filtration or diffusion. In this respect the amniotic fluid proteins appear similar in composition to other interstitial fluids. The sequestered components of amniotic fluid nevertheless show much resemblance in distribution to that of fetal serum. Abbas and Tovey (1960) demonstrated the similarity of electrophoretic patterns of amniotic fluid proteins and those of maternal serum which had been dialyzed across fetal membranes. Despite these observations, the general electrophoretic pattern of human amniotic fluid proteins resembles that of cord serum rather than maternal serum, leading Brzezinski et al. (1961) to question the possible maternal origin of amniotic fluid proteins.

Usatequi-Gomez et al. (1966) showed, by a combination of acrylamide disc electrophoresis and immunodiffusion, that the proteins of the amniotic fluid originated at least partly from maternal serum. It was found that haptoglobin 1 - 1 was present in all amniotic fluid derived from mothers of this type, while it was absent in the corresponding cord sera. Immunoglobulin A was also present only in amniotic fluid and corresponding maternal sera and absent in cord sera. In addition transferrin and ceruloplasmin values approximate those of the maternal rather than those of fetal sera. It was shown in preliminary studies

that any protein present in cord sera was absent in maternal sera and amniotic fluid.

According to Usatequi-Gomez and Morgan (1968), one of the interesting protein groups in amniotic fluid is the group specific Gc protein which is genetically determined. The mother and fetus were found to belong to different Gc types. The Gc type of the amniotic fluid was found to be similar to the maternal Gc type and thus showed that the source of this protein was the maternal host. These findings, together with previous work of the author, strongly suggested that all proteins in the amniotic fluid are of maternal origin.

Rvoslahti et al. (1966) also showed that the Gc protein of amniotic fluid is obtained from the maternal circulation. Taken in conjunction with previous investigators (Abbas and Tovey, 1960; Dancis et al., 1960; Brambell et al., 1952) proved the fact that human placental lactogen (HPL) cannot be detected in cord blood even though it is present in amniotic fluid. These results confirm that most of the proteins in the amniotic fluid are of maternal origin.

In human beings there are marked differences between the non-pregnant and maternal serum constitutions (Longsworth et al., 1945). Maternal serum is characterized by decreased proportions of albumin and gamma globulin and increased proportions of alpha and especially beta globulin. The human fetal serum is much closer in composition to the non-pregnant serum than to the maternal serum. Its most noticeable discrepancy is in the decreased proportion of gamma globulin in fetal as compared with the non-pregnant adult serum (Brambell et al., 1952).

Inspection of densitometer curves indicated higher proportions of

albumin to globulin for human beings in amniotic fluid and cord serum than was found in maternal serum (30-42 weeks of gestation). One draw-back was poor resolution of the alpha fraction in specimens of amniotic fluid (Stander et al., 1964).

Moore et al. (1962) observed in human beings that the albumin of maternal sera decreased during pregnancy, whereas alpha and beta globulins, particularly beta globulin, increased greatly and gamma globulin remained essentially unchanged. Almost the opposite condition prevailed in the developing embryo. There was a marked rise in albumin and gamma globulin but the alpha and beta globulins remained at the same low level from three months to parturition.

When Brzezinski et al. (1961) studied the electrophoretic distribution of proteins in amniotic fluid and in maternal and fetal serum collected from 23 women during delivery, they found that the total protein values were lower in amniotic fluid. The relative concentration of albumin in the amniotic fluid was found to somewhat exceed that in fetal serum and was markedly higher than in maternal serum. The percentages of alpha and beta globulin fractions in fetal serum and amniotic fluid were very close, but both were lower when compared with maternal serum. On the other hand, they stated that the relative concentration of gamma globulins in the amniotic fluid was markedly lower than in fetal and maternal serum.

In amniotic fluid the $alpha_1$ globulin fraction appeared to account for a somewhat larger proportion of total protein. However, it followed the albumin zone so closely that it could not be separately determined with sufficient accuracy.

The concentration of beta-lipoprotein in the maternal serum was elevated as compared with the non-pregnant controls and in the newly born considerably decreased, as compared with the mother. No protein bound lipids could be detected in the amniotic fluid even upon fifteen-fold concentrations (Brzezinski et al., 1961).

Total protein content of amniotic fluid in human beings was found to be related to duration of gestation but was noted to be significantly lower in patients in labor than those who were not in labor. The mean total protein value for 15 patients who were in labor at the time amniotic fluid was obtained was 0.23 gm/100 ml, as compared to 0.28 gm/100 ml for patients not in labor (Stander et al., 1964).

Brambell et al. (1952) observed that rabbit fetal serum (at 25 days of gestation) differs in several respects from that of the mother. First, the total protein concentration was much lower, falling between 50 and 60% of that of the mother. Secondly, four components were distinguishable in the fetal sera. The proportions in which these components occurred differ strikingly from those of the maternal sera, but they may be taken to correspond to albumin, alpha, beta and gamma globulins. The beta globulin constituted 43.6% of the total fetal serum protein and only 16.1% of the total maternal serum protein. Moreover, the beta globulins attained a higher absolute concentration (11.1 mg/ml) in the fetal serum as compared to that of the maternal serum (7.3 mg/ml). The albumin and gamma globulin were both relatively and absolutely less in the fetal serum than in the maternal serum.

The amount of amniotic fluid during the gestation period of the rat increased from the 12th to the 18th day of gestation. There was a

decrease in the amount of amniotic fluid during the remaining 4 days of the gestation period (Wirtschafter and Williams, 1957). The total protein of amniotic fluid fluctuated throughout the entire period of gestation. From the 18th day through the 22nd day of gestation, there was a marked increase in all protein components. Unlike serum protein levels, amniotic fluid contained a higher concentration of globulin than albumin until the 21st day of gestation. The albumin concentration increases in greater proportion than the globulin from the 19th day on, so that the concentration of albumin exceeds that of the globulin at the end of the gestation period, the 22nd day (Wirtschafter, 1958). Wirtschrafter and Williams (1957) have indicated that there is a higher concentration of globulin than albumin in the amniotic fluid of rat until the 21st day of gestation, at which time there is a reversal of this ratio. Marsh et al. (1964) have also indicated that the albumin to globulin ratio in amniotic fluid of rats increases during pregnancy.

Characterization of Amino-Acid Composition

Quantitative free amino acid levels of amniotic fluid, maternal and fetal sera, and fetal urine were determined in 9 early human pregnancies. Statistical analysis indicated a positive correlation between amino acid levels in amniotic fluid with those in fetal serum and urine. These data suggested that amniotic fluid and fetal serum, in addition to placental exchanges with fetal urine act as a diluent of amniotic fluid. Analogous studies in 2 pregnancies indicated a higher turnover of amino acids in maternal serum with a slower rate in amniotic fluid (A'Zary et al., 1973).

Reid et al. (1971) quantitatively determined 21 amino acids in

amniotic fluid and maternal plasma of 27 normal women at two periods of normal gestation. The plasma levels of the 21 amino acids were also determined for a group of normal non-pregnant adults. Significant differences were found for levels of plasma amino acids in both 7 to 8-week and 36 to 40-week gestational groups as compared to the non-pregnant group. The free amino acid levels of amniotic fluid did not reflect maternal plasma levels. Sixteen amniotic fluid amino acids were significantly lower at 36 to 40 weeks than 14 to 18 weeks.

It was reported by Wirtschafter (1958) that free amino acids of human amniotic fluid at full-term pregnancy showed species differences and McKay et al. (1955) reported that the amino-nitrogen content of human hydatidiform mole fluid was elevated over that of maternal plasma. Kerr and Kennan (1969) found that in the rhesus monkey the total value of 19 amino acids in the amniotic fluid decreased sequentially with advancing gestational age. The same trend was seen for most of the individual amino acids. A total value of 19 amino acids in maternal and umbilical vein sera was also indicated. The value of fetal serum was consistently elevated over those in both maternal serum and amniotic fluid. The value of maternal serum showed a rapid decrease early in pregnancy which stabilized by 75 days of gestational age. At this point the value of amniotic fluid was comparable to that of the maternal serum. Thereafter it was always below the value of both maternal and fetal sera. Lesinski et al. (1967) also noted high levels of amino acids in the blastocyst fluid of rabbits between the fifth and seventh days after mating; shortly thereafter a noticeable drop occurred in the level of most of the individual amino acids. Wirtschafter (1958) investigated

amniotic fluid throughout the last 8 days of pregnancy in the rat, and noted that amino acid levels rose with increasing gestation. He also noted that several amino acids were found at higher levels in amniotic fluid than in maternal blood.

DMSO: Its Metabolism and Teratological Implications

Most studies on the metabolism of DMSO have indicated that dimethyl sulfide and dimethyl sulfone are the end products of its interaction with other compounds in biological systems. Rammler and Zaffaroni (1967) suggested that one of the chemical properties of DMSO, namely, its ability to be oxidized, could be utilized "nonspecifically" by cells. Ando (1957) demonstrated the ability of bacteria to reduce DMSO to dimethyl sulfide under specific anaerobic conditions. Thus, it can act as a source of oxygen. Also, Wong et al. (1971) found that both man and miniature pigs transformed DMSO to dimethyl sulfone ($DMSO_2$) and dimethyl sulfide (DMS). Dimethyl sulfone was excreted in the urine, whereas DMS was eliminated in the expired air. In man, the relative amounts of DMS and $DMSO_2$ in the plasma were similar to those found in the urine.

Congenital abnormalities induced by DMSO have been reported in mammals, amphibians, and birds. Ferm (1966) reported that DMSO induced gross congenital malformation in hamsters. Marin-Padilla (1966) found that reduced ovulation in rats could be produced by DMSO administration. They suggested that DMSO may have antagonistic effects on gonadotropin. According to Hammerman (1966), DMSO inhibits larval involution and metamorphosis in frogs. Caujolle (1965) and Browne (1968) reported teratological effects of DMSO in the chick. They found that injections of 50% DMSO induced limb bud blisters, allantoic congestion, blood blisters,

blisters of the eyes, anophthalmia, micropthalmia, and coelosomia. Fluid and electrolyte disturbances were also found in embryonic and extra-embryonic compartments of chick embryo (Browne, 1968; 1970).

Dimethylsulfoxide: A Biomedical Tool for Analyzing Changes in Amniotic Fluid Moiety

Dimethylsulfoxide (DMSO) had been shown to cross the dermal barrier rapidly and in high concentration. It accomplished this with little or no permanent tissue damage (Kiligman, 1965). It is not unreasonable to assume that this visibly innocuous permeation of DMSO though a protein barrier, is the result of reversible configurational changes of these proteins because of water substitution by DMSO (Rammler and Zaffaroni, 1967). The enzyme, lysozyme, has been shown in DMSO to assume a configuration having greater flexibility than the native protein without any change in the molecular weight (Hamaguchi, 1964). In these studies, it was observed that the major configurational changes occurred with DMSO concentrations between 60 to 70%. This effect was clearly shown in rotatory dispersion studies with the enzyme lysozyme. All of these measured changes were completely reversible. In protein typographical studies by different spectra (Herskovits and Laskowshi, 1962), solvent induced shifts in the absorption maxima have been found. These shifts in absorption maxima of amino acids such as tyrosine and tryptophan have been attributed to changes in the special environment that surrounds these groups in the native protein. The extent of these spectral changes in different solvents is a measure of the exposure of these groups in the protein to the solvent. In studies with lysozyme, there is a gradual increase in the exposure of buried tryptophanyl residues

with increased DMSO concentrations - this change increasing abruptly again at 70% DMSO (Hamaguchi, 1964). The effectiveness of DMSO as a spectral perturbant is related not only to its special chemical characteristic, but also to its size. In studies with bovine serum albumin under carefully defined conditions, DMSO was a more effective perturbant than ethylene glycol, polyethylene glycol, glycerol, and sucrose (Herskovits and Laskowshi, 1962). Despite its difference in chemical structure, these authors indicated that there was a correlation between the size of the solvent and its effectiveness as a perturbant with albumin. It was suggested that, because of its comparatively small size, DMSO was able to penetrate regions of the protein subunits interfaces more readily than the other bulkier solvents. This effect depended upon the location of the chromophore and was not found for all proteins (Williams et al., 1965).

Thus, DMSO appears to be extremely effective in altering the configuration of protein, and this change is apparently reversible with several other enzymes (Rammler, 1966). Its effectiveness in this regard appears to be related to its size (Barner, 1965) and capacity to substitute for, or bind, water, in addition to affecting other hydrogen bonded structures. These observations suggest a possible mode of action of DMSO in the living system (Rammler and Zaffaroni, 1967).

fetal-matched gravid rats and the cells and serum separated by collecting the blood in microfuge tubes and centrifuging for 10 min at 3000 rpm. All separated amniotic fluid and blood serum was either prepared for assay immediately or frozen until time of use. Some female rats were injected intraperitoneally with 0.25 ml of 75% dimethylsulfoxide (DMSO) on 14-1/2 days of gestation.

Electrophoretic Preparations and Analyses

Cellulose-acetate microelectrophoresis

Gelman high resolution buffer (pH 8.8), Ponceau S, rinse solution (5% acetic acid) and Sepra Clear solution were prepared and respective trays were filled with 100 ml of each solution.

Sepraphore III (cellulose acetate strips) were submerged in Tris-Barbital Buffer (pH 8.8) and allowed to soak for at least 10 min. Fresh buffer was then added to the Sepra-Tek electrophoretic chamber and the buffer levels equalized in the two electrode compartments. The Sepraphore III strips were removed from the buffer soaking tray and placed on absorbent pads. Samples were immediately prepared for application and applied to the etched areas on the sample wells with capillary pipets. The applicator keys were then depressed in the samples for 15 sec, released and left in position.

The acetate strips were blotted on the absorbent pad lightly with a second pad and carefully placed on the membrane frame assembly. The loaded frame assembly was then placed in the chamber and immediately covered. The loaded applicator was positioned on the chamber cover and carefully, but firmly, keys were depressed. The keys were held in contact with the membrane for approximately 20 sec and then released.

CHAPTER III

MATERIALS AND METHODS

Maternal serum, fetal serum, and amniotic fluid were acquired for electrophoretic analysis and total protein determination by housing female Long-Evans rats in proestrous with males of the same strain overnight. The day of conception was determined by the vaginal smear technique. This was done by removing the male rat from the cage of the female the next morning and making vaginal smear preparations. The preparations were then observed microscopically for sperm and, if present, this was considered day 1/2 of conception. The pregnancy was then allowed to continue until the day amniotic fluid and fetal serum were to be extracted.

On pre-determined days (13-1/2 - 20-1/2) amniotic fluid and fetal serum were acquired from fetuses of gravid female rats by exposing them to anesthesia with ethyl ether. While the rats were under anesthesia, an incision was made into the abdominal wall, exposing the uteral horns, which were subsequently excised.

Amniotic fluid was removed from the amniotic sac by aspirating the fluid with a technique devised by Browne (1968). The samples were subsequently centrifuged for 5 min to separate fluids and cellular debris. The fetal blood was acquired by removing the chorionic and amniotic membranes and puncturing the anterior cardinal veins or neighboring peripheral veins on the fetus. The blood samples were immediately centrifuged for 10 min and the serum collected.

Maternal blood serum was acquired by heart-puncture from maternal

The safety cap was immediately plugged onto electrodes, the power supply turned on, and the voltage adjusted to 200. The samples were electrophoresed for 30 min.

Immediately after the power was shut off from the electrophoretic chamber, the acetate strips were carefully removed from the frame assembly and floated onto the surface of Ponceau S in the staining tray. When the strips were completely saturated with Ponceau S, they were completely submerged and allowed to stain for 10 min. The Sepraphore III strips were removed from the Ponceau S and placed in the first of three rinse solutions and agitated. The strips were then taken through two more consecutive trays of rinse solution until the background of the strips was translucent.

The strips were removed from the final rinse tray and laid lengthwise on glass slides, being careful not to trap air under the membranes. A second glass slide was pulled lightly across the surface to remove excess fluid. The ends of the strips were folded over the edge of slides and the excess was trimmed, allowing approximately 1/4 of the strip to overlap. The strips on the glass were placed in the Sepra Clear solution and allowed to soak for 1-1/2 min. The strips were then removed from the Sepra Clear and again excess fluids were removed with a second slide. The slides were heated in a 90 C oven for 10 min so that the background could become completely transparent. They were removed from the oven and allowed to cool and the backs of the slides were cleaned with alcohol. The slides were then read on a Digiscreen M scanning densitometer (Gelman Instruments, Inc.), at 550 nm using a green filter. The scanning results were recorded graphically on a Digiscreen Recorder.

Cyanogum-41 gel electrophoresis

A Tris-Na_2EDTA-Borate buffer (0.18 M), pH 8.4 was used as a electrophoresing medium. The gels were made up from a 5% cyanogum-41 (95% acrylamide and 5% bisacrylamide) solution. The cyanogum was dissolved in the buffer and 0.1 ml of tetramethylethylenediamine (TMED) was added for every 100 ml of gel solution. Immediately prior to pouring the gel into the glass tubes, 100 mg of ammonium persulfate (AP) was added for every 100 ml of gel solution to induce rapid polymerization. About 2 ml of this solution was injected into 5 mm X 80 mm glass tubes in the final preparation for receiving samples for electrophoresing. For amniotic fluid, maternal serum and fetal serum, 60, 2.5, and 10 microliters of sample were used, respectively. An equal volume of 40% sucrose was mixed with each sample to increase the density so that the sample would settle evenly on top of the gel columns. A small amount of bromophenol blue was added to each sample mixture to serve as a tracking dye during the electrophoretic separation. The outer compartment of the Gel Cell unit (Gelman Instruments, Inc.) was filled with 700 ml of buffer and the inner tank was filled with 300 ml of buffer after the gel-containing tubes were put in the chamber. The sample mixture was added to the tubes and the power supply current was set at 4 ma per tube. Separation was continued until the tracking dye had migrated to the end of the tubes, or for 2 hr 15 min. The gels were then removed from the glass columns, put in test tubes and stained in 0.25% amido black 10B made up in a methanol-water-acetic acid solution (5:5:1) for 30 min. The gels were destained in 7% acetic acid. Gels were then quantitated on the Gelman Digiscreen-M using a blue-violet filter at a wavelength of 600 nm.

Total protein determination

Preparatory tubes were labeled as reagent blank, standard, and sample. To the reagent blank tube, 0.1 ml of total protein blank was added; 0.1 ml of protein standard was added to the standard tube; and to sample tubes 0.1 ml of sample was added. This was followed by the addition of 5 ml of biuret reagent to all tubes. The solution was mixed and allowed to stand for 15 min at room temperature (Sigma). The tubes were then read on a Turner spectrophotometer at 540 nm using the reagent blank as a reference source. Calculations were made using the following formula (Gornall et al., 1949).

$$\text{Serum Total Proteins (gm/100 ml)} = \frac{\text{A Sample}}{\text{A Standard}} \times 8.0$$

(in which A stands for absorbance)

Flame photometry

An Instrumentations Laboratories (IL) 343 Digital Flame-Photometer was used for all sodium and potassium assays. The flame-photometer was zeroed in with a lithium standard (4000 mEq/L) that was diluted twenty times with deionized water. The instrument was standardized using IL sodium and potassium standards (140 mEq/L and 5 mEq/L). In order to determine the amounts of sodium and potassium in samples of maternal serum, fetal serum, and amniotic fluid, 5 microliters of each were added to 1 ml of diluted lithium blank and the samples were mixed and recorded on the digital flame-photometer.

CHAPTER IV

RESULTS

Total Protein

In the Long-Evans rat, we have observed that the total protein of maternal serum fluctuates from days 13-1/2 - 20-1/2. It was observed to be highest on day 14-1/2 (7.13 g/100 ml) and lowest on day 19-1/2 (5.86 g/100 ml) (Table 1, Fig. 1).

There was a gradual increase in the total protein of amniotic fluid from day 13-1/2 - 20-1/2. The exception in this general pattern occurred on day 15-1/2, wherein analysis showed a slight drop in the total protein to 0.1029 g/100 ml on day 14-1/2 (Table 2). Likewise, that in the blood serum of fetuses on 13-1/2 to 20-1/2 days of gestation there was a gradual increase in total protein. The total protein ranged from 0.402 g/100 ml on day 13-1/2 to 2.419 g/100 ml on day 20-1/2 of gestation (Table 3). A graph showing the comparative changes in fetal serum and amniotic fluid total protein is illustrated in Fig. 2.

Cellulose Acetate Electrophoresis

In an attempt to characterize the proteins in maternal serum, fetal serum and amniotic fluid, electrophoresis was first carried out using cellulose acetate strips. Electrophoresis was performed on fetal serum, maternal serum, and amniotic fluid from 13-1/2 to 18-1/2 days of gestation. In maternal serum we observed that during the overall fetal gestation period there is only a very slight change in albumin concentration as evidenced by comparison with blood serum from non-pregnant female

Table 1. Total protein changes in maternal serum.

Day	Number of Samples	Mean	S.D.	Range
13-1/2	17	6.848	± .662	5.5 - 7.9
14-1/2	12	7.139	± .62	6.4 - 8.3
15-1/2	13	7.044	± .239	5.4 - 8.7
16-1/2	10	6.47	± .238	6.7 - 6.2
17-1/2	12	6.506	± .195	6.3 - 6.7
18-1/2	8	5.742	± 1.16	5.0 - 6.79
19-1/2	10	5.86	± .596	5.09 - 6.5
20-1/2	8	5.87	± .209	5.6 - 6.1

Fig. 1. A graph showing changes in total protein of maternal serum from 13-1/2 - 20-1/2 days of gestation.

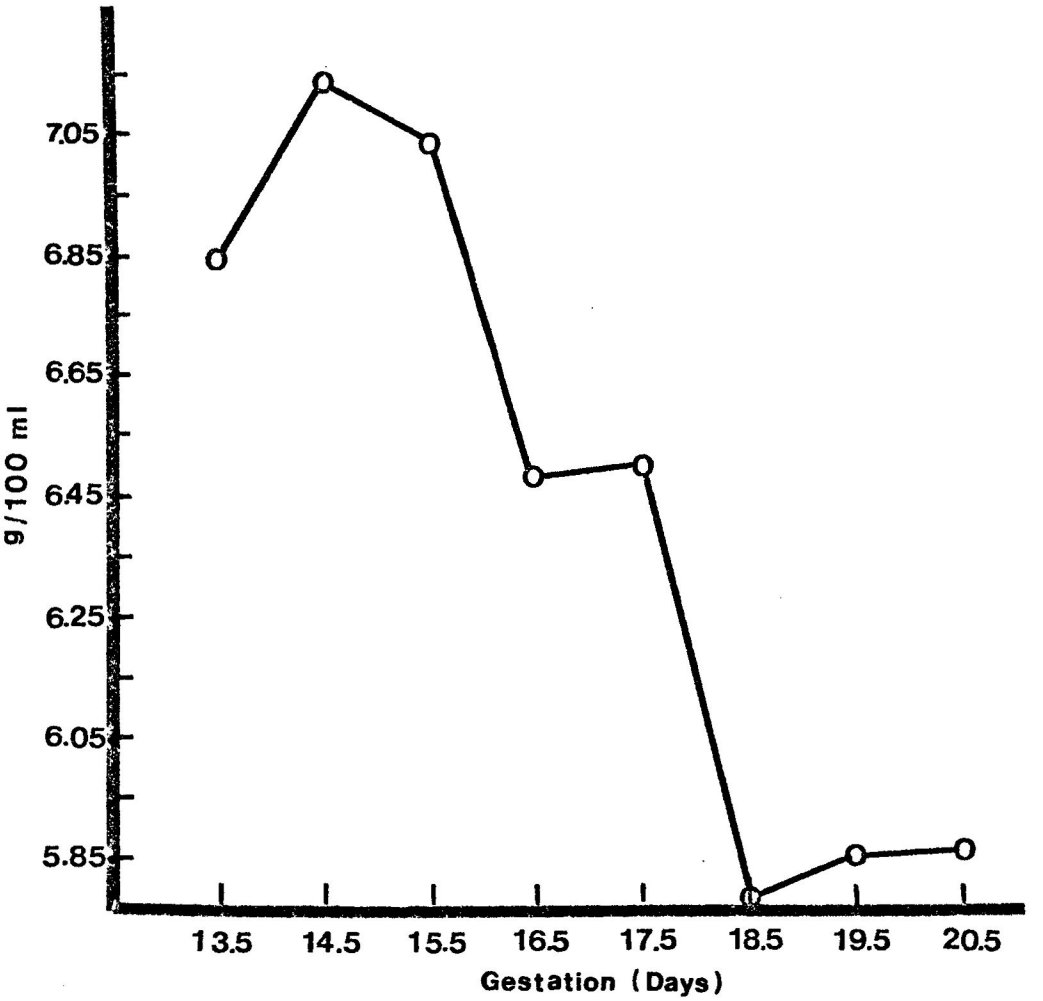

7.05
6.85
6.65
6.45
6.25
6.05
5.85
g/100 ml
13.5
14.5
15.5
16.5
17.5
18.5
19.5
20.5
Gestation (Days)

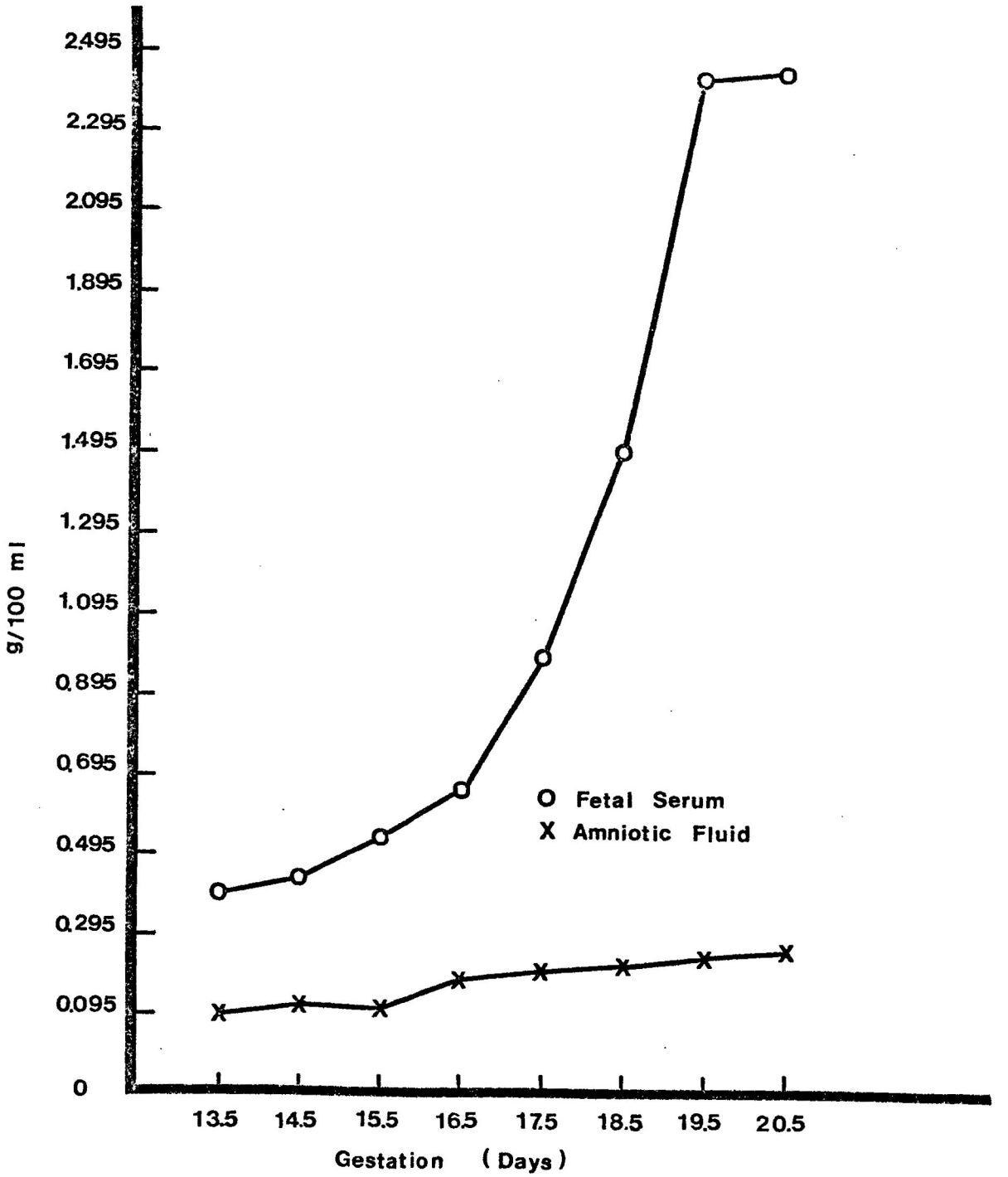
Table 2. Total protein changes in amniotic fluid of fetal rats from 13-1/2 - 20-1/2 days old.

Day	Number of Samples	Mean	S.D.	Range
13-1/2	13	0.09536	± .0216	.062 - .114
14-1/2	13	0.113	± .02816	.073 - .160
15-1/2	15	0.1029	± .0304	.045 - .170
16-1/2	16	0.177	± .0332	.145 - .200
17-1/2	11	0.1901	± .0255	.160 - .237
18-1/2	17	0.2100	± .400	.073 - .233
19-1/2	10	0.2306	± .048	.189 - .305
20-1/2	8	0.2456	± .090	.187 - .340

Table 3. Total protein changes in fetal serum from days 13-1/2 - 20-1/2 of gestation.

Day	Number of Samples	Mean	S.D.	Range
13-1/2	3	0.402	± .233	.360 - .420
14-1/2	3	0.443	± .256	.364 - .490
15-1/2	5	0.547	± .250	.364 - .600
16-1/2	6	0.669	± .240	.547 - .672
17-1/2	10	1.048	± .235	1.016 - 1.36
18-1/2	8	1.495	± .235	1.226 - 1.6
19-1/2	10	2.415	± .9020	1.06 - 3.619
20-1/2	8	2.419	± .611	1.846 - 3.2

Fig. 2. A graph comparing total protein of fetal serum and amniotic fluid.


2.495
2.295
2.095
1.895
1.695
1.495
1.295
1.095
0.895
0.695
0.495
0.295
0.095
0
g/100 ml
O Fetal Serum
X Amniotic Fluid
13.5
14.5
15.5
16.5
17.5
18.5
19.5
20.5
Gestation (Days)

rats (Table 4). The alpha-1 fraction of maternal blood serum, however, gradually decreased during the 13-1/2 to 18-1/2 day period of fetal gestation. The other three fractions, alpha-2, beta, and gamma, fluctuated markedly during the same period (Table 4).

Analysis of the fetal serum showed that the prealbumin fraction did not separate from the albumin fraction in samples from 13-1/2 - 15-1/2 day-old fetuses. However, the albumin concentration for these days was higher than on days 16-1/2 - 18-1/2. There was a gradual decrease in albumin fraction serum samples of fetal blood examined on 16-1/2 to 18-1/2 days of gestation. This decrease occurred concomitantly with a gradual increase in the serum prealbumin fraction (Table 5). The percent of globulins fluctuated during the gestation period. In particular, it was noted that the gamma globulin was higher on 13-1/2 and 14-1/2 days of gestation than at any other period (Table 5).

As for the amniotic fluid only two fractions, prealbumin and albumin, could be determined with any degree of accuracy. Densitometric scanning allowed for the detection of only traces of beta globulin. At days 13-1/2, 16-1/2, and 18-1/2, over 50% of the protein in the amniotic fluid was prealbumin and albumin (Table 6).

Gel Electrophoresis Analyses

The protein fractions in maternal serum fluctuated from 13-1/2 - 20-1/2 days of fetal gestation. There is very little change in the albumin fraction which ranges from 31-32%. The remaining fractions represented about 69% of the total protein (Table 7). These fractions fluctuated comparatively more than the albumin fractions. During the fetal gestational period nearly all of the maternal serum globulins are

Table 4. Summary of electrophoretic distribution of proteins in maternal serum on cellulose acetate strip.*

Day	Albumin	Alpha-1	Alpha-2	Beta	Gamma
13-1/2	44.6	9.3	15.15	20.8	9.7
14-1/2	39.8	12.3	15.9	19.6	12.4
15-1/2	40.0	12.0	16.0	19.0	13.0
16-1/2	45.0	10.2	17.9	18.9	7.6
17-1/2	42.5	9.9	19.9	17.4	10.5
18-1/2	41.2	10.0	19.5	19.9	9.6
NPS**	45.8	8.6	18.46	14.35	12.7

* Expressed in relative percentages: the sum of the total fractions divided by each individual fraction multiplied by 100.

** NPS = Non-pregnant serum

Table 5. Summary of electrophoretic distribution of proteins in fetal serum on cellulose acetate strip.*

Day	Prealbumin	Albumin	Alpha	Beta	Gamma
13-1/2	-	40.0	22.7	16.8	20.5
14-1/2	-	40.0	21.7	17.8	20.5
15-1/2	-	48.0	29.2	14.6	9.0
16-1/2	11.0	33.0	22.0	18.0	16.0
17-1/2	15.9	28.7	24.7	19.0	11.8
18-1/2	23.0	26.9	28.5	13.8	8.5

* Expressed in relative percentages.

Table 6. Summary of electrophoretic distribution of proteins in amniotic fluid on cellulose acetate strip.*

Day	Prealbumin	Albumin	Alpha	Beta	Gamma
13-1/2	27.0	25.5	21.2	11.6	14.6
16-1/2	23.9	31.0	19.6	14.6	10.0
18-1/2	23.5	39.9	12.5	14.6	8.5

* Expressed as relative percentages.

Table 7. Summary of densitometric analyses of maternal serum proteins from gel electrophoresis in relative percent.

Day		Albumin	Alpha-1	Alpha-2	Alpha-2[1]	Transferrin	Beta	Gamma-1	Gamma-2
13-1/2	M.	31.0	8.4	8.0	12.8	13.0	12.2	14.6	
	S.D.	±2.4	±1.1	±1.4	±1.3	±2.2	±2.3	±2.0	
	R.	28.0	7.5 - 8.7	5.8 - 11.0	10.5 - 15.0	10.0 - 17.0	9.5 - 16.5	12.6 - 20.0	
14-1/2	M.	32.4	5.6	7.4	15.5		17.2	16.4	5.1
	S.D.	±1.5	±1.1	±0.92	±1.7		±2.6	±2.1	±1.5
	R.	30.4 - 34.1	4.2 - 8.1	6.0 - 8.5	9.0 - 18.53		10.0 - 20.0	14.1 - 20.8	3.0 - 8.3
15-1/2	M.	30.6	6.3	6.5	13.3	8.0	15.0	15.0	5.1
	S.D.	±3.0	±0.7	±2.0	±1.4	±1.9	±3.1	±4.9	±0.6
	R.	26.5 - 35.0	5.0 - 7.4	4.6 - 10.5	12.0 - 16.0	6.0 - 12.0	10.0 - 18.6	14.6 - 20.6	4.5 - 6.0
16-1/2	M	32.7	5.3	6.3	10.8	6.4	19.0	14.8	5.0
	S.D.	±2.9	±1.2	±1.2	±1.5	±1.9	±1.7	±2.2	±2.1
	R.	27.7 - 36.0	4.1 - 6.6	4.4 - 8.1	9.5 - 13.4	4.5 - 9.6	16.6 - 21.7	3.6 - 18.4	3.4 - 9.0
17-1/2	M.	30.0	4.6	5.8	10.8	5.5	18.7	19.0	5.6
	S.D.	±2.5	±0.8	±0.6	±1.1	±0.6	±3.8	±1.9	±0.4
	R.	26.1 - 32.0	3.7 - 5.5	4.7 - 6.3	9.2 - 11.7	4.4 - 7.0	14.2 - 22.2	17.0 - 21.7	5.0 - 6.1
18-1/2	M.	31.2	5.5	8.5	9.6	16.3	16.8	4.0	8.1
	S.D.	±2.6	±1.1	±1.2	±1.8	±4.1	±1.4	±1.2	±1.3
	R.	28.8 - 35.4	3.7 - 6.4	7.0 - 10.42	7.5 - 12.1	7.1 - 9.8	10.0 - 22.6	14.0 - 18.6	3.0 - 6.0
19-1/2	M.	32.0	6.6	7.8	8.2	8.4	16.0	17.5	3.5
	S.D.	±3.5	±0.7	±2.7	±4.1	±1.5	±1.4	±1.1	±0.8
	R.	29.0 - 35.8	4.6 - 6.2	5.4 - 11.0	6.0 - 15.6	6.0 - 10.0	15.2 - 17.0	17.0 - 19.3	2.7 - 5.0

Table 7 (continued)

		Albumin	Alpha-1	Alpha-2	Alpha-2[1]	Transferrin	Beta	Gamma-1	Gamma-2
20-1/2	M.	30.6	5.0	5.7	12.0	6.0	14.0	22.0	4.7
	S.D.	±1.5	±1.2	±0.6	±1.0	±1.9	±1.4	±1.9	±2.1
	R.	30.4 - 34.1	4.1 - 6.7	4.7 - 6.3	10.5 - 14.5	4.5 - 9.6	12.0 - 16.0	19.0 - 23.0	3.4 - 8.0
NPS	M.	32.0	4.7	4.8	11.9	6.6	19.1	16.0	4.9
	S.D.	±3.6	±1.7	±1.1	±1.8	±1.2	±0.5	±1.5	±0.7
	R.	29.5 - 36.1	3.5 - 6.5	3.6 - 5.7	10.1 - 13.8	5.3 - 7.8	18.5 - 19.6	15.0 - 18.1	4.2 - 5.7

M = Mean

S.D. = Standard Deviation

R = Range

NPS = Non-pregnant serum

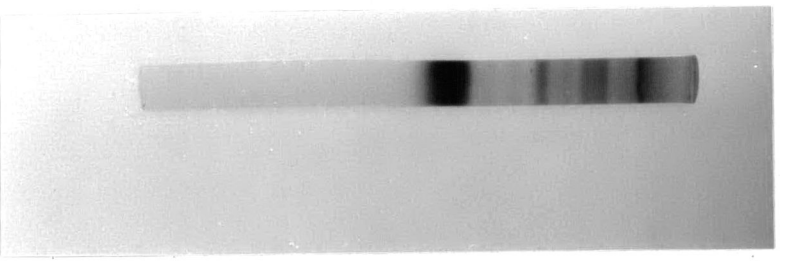
elevated above those in the non-pregnant serum except for transferrin (Table 7). Figure 3 shows a polyacrylamide gel profile and typical densitometric scan of maternal serum on 19-1/2 days of gestation.

Figure 4 shows a polyacrylamide gel fraction profile and typical densitometric analysis of fetal serum on 19-1/2 days of gestation. Six bands are detected in fetal serum during this period of gestation. In fetal serum the prealbumin fraction fluctuates from a high of 18% on 13-1/2 days to a low of 8.5% in the 14-1/2 day fetuses. Subsequently in the 15-1/2 and 16-1/2 day fetuses this specific fraction (prealbumin) increases markedly to 13%, and in the 17-1/2 through 20-1/2 day fetuses again there was exhibited a small but detectable fluctuation in concentration. In the 20-1/2 day fetuses prealbumin is highest (31%)(Table 8). The albumin fraction was found to be at its lowest concentration in the fetal serum of 20-1/2 day fetuses.

In the fetal rat blood serum the lowest alpha fraction concentration was observed on days 17-1/2 and 18-1/2 of gestation. The lowest beta fraction concentration was obtained on 17-1/2 days; the lowest gamma fraction concentration on 13-1/2 days of gestation (Table 8). The highest beta and gamma fraction concentrations occurred on the 14-1/2 day and the highest concentration of the alpha fractions in 19-1/2 day fetal serum (Table 8). In Fig. 5 gel profiles are shown for 15-1/2 - 20-1/2 days fetal serum. As many as 8 bands could be separated in fetal serum. We observed that in amniotic fluid of rat fetuses the concentration of prealbumin and albumin fractions represented more than 50% of the total protein (Table 9). The albumin concentration fluctuated during the gestation periods from a low of 31.4% at 13-1/2 days

Fig. 3. Polyacrylamide gel profile and densitometric scan for maternal serum.

T = transferrin



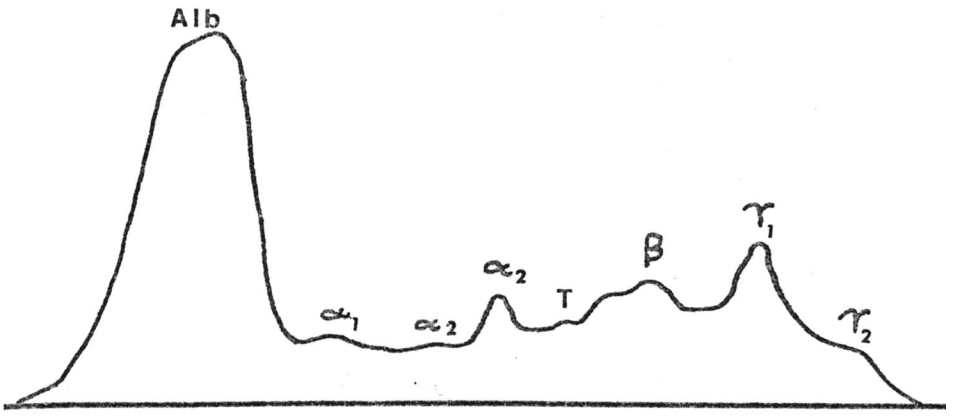
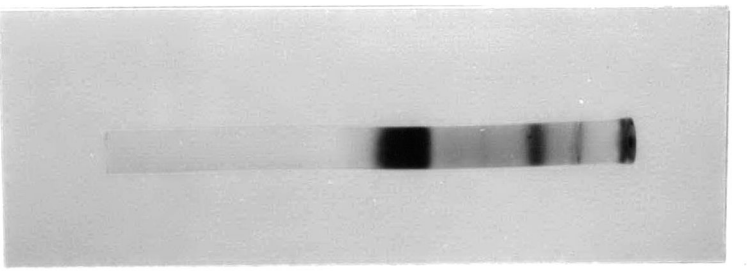

Alb
α1
α2
α2
T
β
γ1
γ2

Fig. 4. Polyacrylamide gel profile and typical densitometric scan of fetal serum on 19-1/2 days of gestation.



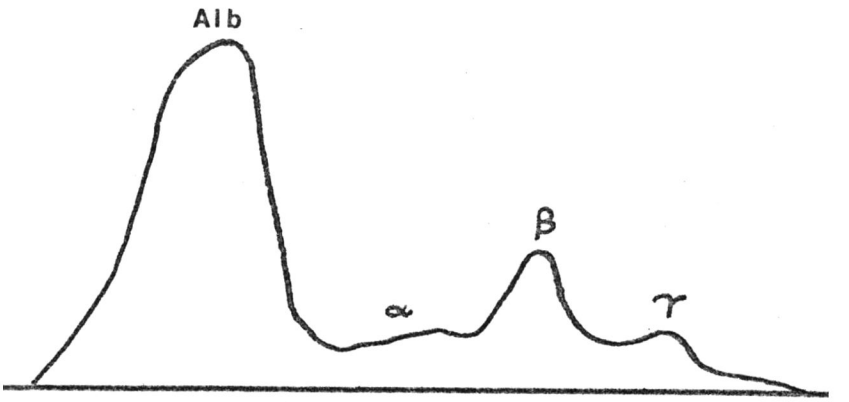

Alb
α
β
γ

Table 8. Electrophoretic distribution of proteinsin fetal blood serum with gel electrophoresis.

Day		Prealbumin	Albumin	Alpha	Beta	Gamma
13-1/2	M.	18.0	40.6	16.5	17.5	7.4
	S.D.	±2.0	±0.848	±0.707	±0.707	±0.565
	R.	17.0 - 19.0	40.0 - 41.0	16.0 - 17.0	18.0 - 17.0	7.0 - 7.8
14-1/2	M.	8.5	42.7	14.43	19.8	14.2
	S.D.	±4.5	±5.9	±5.6	±5.42	±6.238
	R.	7.0 - 13.6	37.3 - 47.3	8.6 - 17.0	15.0 - 25.7	10.0 - 21.4
15-1/2	M.	13.8	43.4	16.83	16.0	10.15
	S.D.	±7.4	±1.75	±5.12	±3.7	±2.74
	R.	13.0 - 23.0	40.8 - 44.8	14.0 - 20.0	10.8 - 19.6	8.2 - 14.2
16-1/2	M.	13.85	43.85	14.47	15.83	11.1
	S.D.	±1.61	±2.54	±1.43	±2.87	±2.5
	R.	12.0 - 15.4	40.0 - 45.0	12.6 - 16.0	13.0 - 19.3	9.0 - 13.5
17-1/2	M.	20.5	44.0	12.2	14.8	8.9
	S.D.	±2.2	±4.0	±1.8	±2.0	±1.69
	R.	18.3 - 23.3	38.0 - 46.7	12.5 - 14.0	12.5 - 17.1	7.8 - 11.0

Table 8 (continued)

Day		Prealbumin	Albumin	Alpha	Beta	Gamma
18-1/2	M.	24.5	39.1	12.5	15.5	8.4
	S.D.	±2.0	±1.08	±2.2	±0.204	±0.048
	R.	23.4 - 27.4	37.6 - 40.1	10.7 - 15.5	15.0 - 16.0	7.0 - 9.6
19-1/2	M.	23.1	31.2	17.8	16.3	11.7
	S.D.	±2.2	±6.2	±3.5	±0.986	±2.06
	R.	23.0 - 25.6	22.0 - 40.9	13.0 - 19.0	14.5 - 17.0	8.7 - 11.7
20-1/2	M.	31.0	26.0	14.0	18.0	11.0
	S.D.	±6.5	±7.07	±7.93	±2.7	±2.23
	R.	27.0 - 36.2	21.0 - 31.0	7.9 - 18.0	15.8 - 20.8	9.2 - 13.5

* Expressed as relative percentage

M = Mean

S.D. = Standard Deviation

R = Range

Fig. 5. Polyacrylamide gel profiles of fetal serum for 15-1/2 - 20-1/2 days, respectively (A-F).

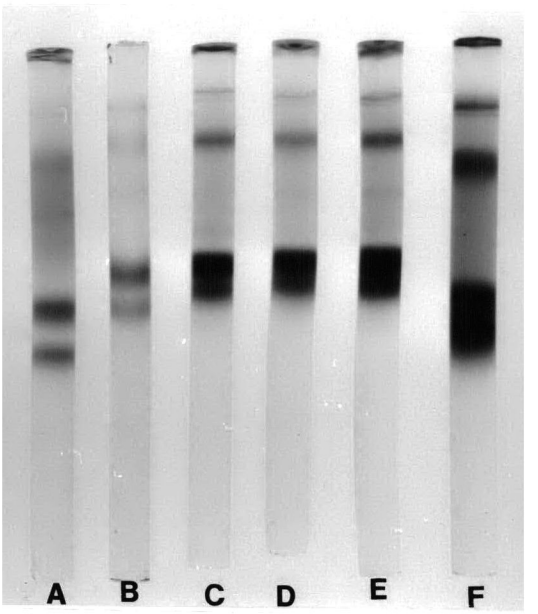

A
B
C
D
E
F

Table 9. Acrylamide gel electrophoretic analyses of amniotic fluid protein fractions in relative percent.

Day		Prealbumin	Albumin	Alpha	Beta	Gamma
13-1/2	M.	30.4	31.4	18.5	10.4	9.7
	S.D.	±3.1	±5.93	±4.7	±2.2	±1.0
	R.	27.4 - 33.6	26.4 - 38.0	7.3 - 10.0	9.0 - 13.0	8.2 - 10.0
14-1/2	M.	19.5	41.5	15.7	12.7	10.6
	S.D.	±4.6	±2.4	±5.9	±2.1	±2.1
	R.	10.6 - 26.9	37.3 - 43.2	13.0 - 18.1	10.1 - 17.1	8.0 - 13.6
15-1/2	M.	20.6	33.6	17.6	13.5	14.7
	S.D.	±3.3	±6.2	±2.7	±1.7	±2.5
	R.	17.0 - 24.8	27.1 - 42.2	14.9 - 20.1	11.8 - 15.1	11.0 - 17.7
16-1/2	M.	15.0	48.7	11.85	13.9	10.0
	S.D.	±3.4	±3.4	±3.4	±1.9	±2.2
	R.	9.9 - 21.0	46.2 - 51.9	8.4 - 18.8	11.0 - 15.5	8.1 - 13.3

Table 9 (continued)

Day		Prealbumin	Albumin	Alpha	Beta	Gamma
17-1/2	M.	14.0	55.0	11.0	12.0	9.0
	S.D.	±3.1	±4.9	±2.8	±1.6	±2.2
	R.	8.7 - 19.2	49.8 - 55.9	7.7 - 18.8	10.8 - 16.1	7.4 - 11.2
18-1/2	M.	14.5	51.5	8.7	12.5	12.8
	S.D.	±1.6	±7.7	±1.3	±0.5	±1.1
	R.	12.5 - 15.5	41.0 - 57.1	7.4 - 10.1	11.4 - 12.5	9.7 - 11.1
19-1/2	M.	27.5	30.5	15.5	14.5	12.0
	S.D.	±4.6	±4.6	±2.9	±1.6	±3.0
	R.	23.5 - 33.0	24.0 - 35.0	12.7 - 18.8	14.0 - 16.1	9.3 - 15.7
20-1/2	M.	34.2	31.2	7.8	16.8	10.0
	S.D.	±2.8	±0.1	±0.7	±1.4	±0.3
	R.	32.0 - 36.2	31.0 - 31.0	7.0 - 8.2	15.8 - 17.8	9.2 - 10.2

M. = Mean

S.D. = Standard Deviation

R. = Range

to 55% on 17-1/2 days, and then again decreases to approximately 31% on day 20-1/2 (Table 9). The concentration of the alpha fraction is lowest on 20-1/2 days; the beta fraction is lowest on 13-1/2 days, and the gamma fraction is lowest on 17-1/2 days of gestation (Table 9).

Figure 6 shows a polyacrylamide profile and densitometric scan of amniotic fluid proteins from 13-1/2 day-old fetuses. Six characteristic bands can be seen. Polyacrylamide gel profiles of protein in amniotic fluid of 13-1/2 and 14-1/2 day-old fetuses are presented in Fig. 7. Figure 8 shows a gel profile of amniotic fluid proteins from 15-1/2 - 20-1/2 day-old fetuses.

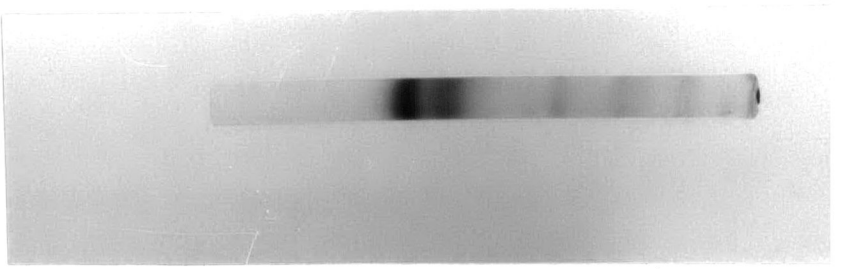
Sodium and Potassium Analyses of Fluids from Normal Rats

In the Long-Evans gravid rat we observed that blood serum sodium concentration was highest on the 13-1/2 day of gestation (147.7 mEq/L) and lowest on 19-1/2 days of gestation (125.5 mEq/L). The potassium concentration in the same fluids was found to be highest on 20-1/2 days (6.45 mEq/L) and lowest on 17-1/2 days (3.9 mEq/L). In the non-pregnant serum the sodium concentration was 152.7 mEq/L (Table 10).

In fetal serum we found that at no time during the gestation period (13-1/2 - 20-1/2 days) did the mean sodium concentration drop below 140 mEq/L. Potassium on the other hand fluctuated markedly and was lowest in 14-1/2-day fetal blood serum (13 mEq/L) (Table 11).

In the amniotic fluid of rat fetuses the sodium and potassium concentrations were lower than those in the fetal serum (Table 12). However, the highest concentration of sodium was observed to be on 19-1/2 days of gestation (141 mEq/L); the lowest concentration (122 mEq/L) was on day 18-1/2 of gestation (Table 12). The highest potassium

Fig. 6. Polyacrylamide gel profile and typical densitometric scan for amniotic fluid from 13-1/2 day-old fetuses.



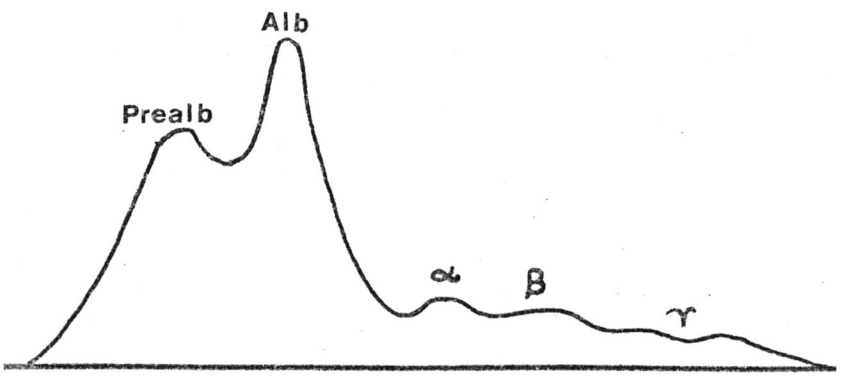

Alb
Prealb
α
β
γ

Fig. 7. Polyacrylamide gel profiles of amniotic fluid for 13-1/2 days (A) and 14-1/2 days (B).

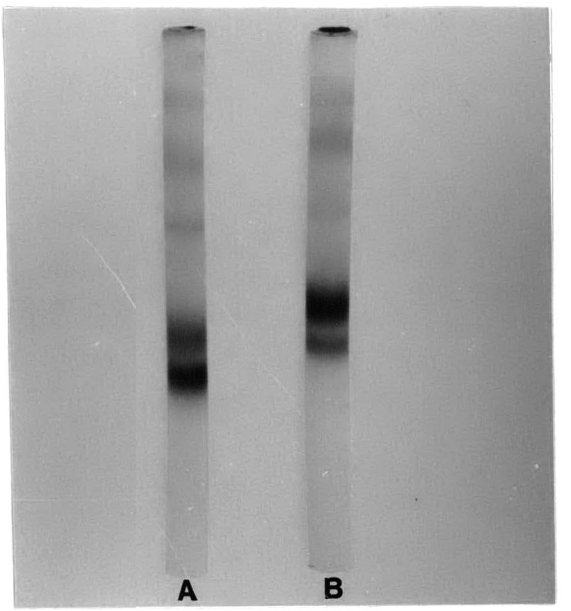

A
B

Fig. 8. Polyacrylamide gel profiles of amniotic fluid for 15-1/2 - 20-1/2 days, respectively (A-F).

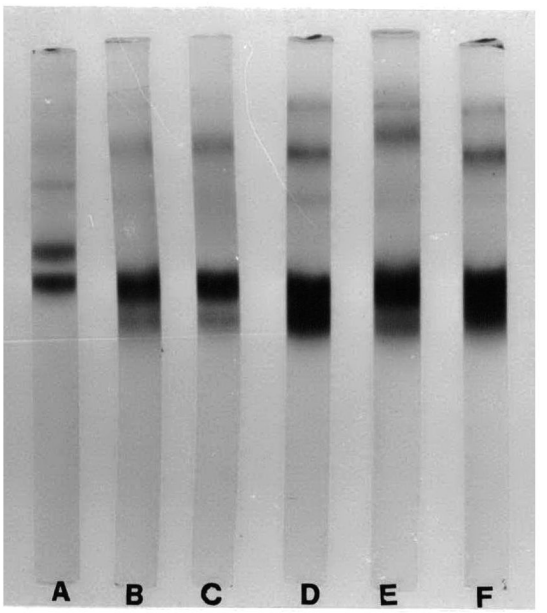

A
B
C
D
E
F

Table 10. Concentrations of sodium and potassium in maternal blood serum on 13-1/2 - 20-1/2 days of gestation.

Day	Number of Samples		Na^+ (mEq/L)	K^+ (mEq/L)
13-1/2	17	Mean	147.7	6.1
		S.D.*	±15	±.79
		Range	127.2 - 151.0	4.8 - 6.8
14-1/2	17	Mean	137.0	5.6
		S.D.	±12	±0.4
		Range	120.0 - 159.0	5.2 - 6.9
15-1/2	10	Mean	135.0	5.6
		S.D.	±10.9	±0.4
		Range	128.0 - 151.0	5.5 - 6.0
16-1/2	10	Mean	135.0	4.0
		S.D.	±10	±0.4
		Range	112.4 - 144.0	3.4 - 4.6
17-1/2	10	Mean	131.8	3.9
		S.D.	±7.9	±0.4
		Range	120.0 - 142.6	3.5 - 4.5
18-1/2	8	Mean	146.3	5.9
		S.D.	±4.6	±0.14
		Range	143.0 - 149.0	5.8 - 6.0
19-1/2	10	Mean	125.5	5.7
		S.D.	±6.7	±0.7
		Range	116.0 - 133.0	4.8 - 6.6

Table 10 (cont'd).

Day	Number of Samples		Na^+ (mEq/L)	K^+ (mEq/L)
20-1/2	8	Mean	127.9	6.45
		S.D.	±0.07	±0.2
		Range	127.2 - 128.1	6.3 - 6.6
NSP**	10	Mean	152.7	6.2
		S.D.	±5.32	±0.6
		Range	145.5 - 159.5	5.4 - 7.0

* S.D. = Standard Deviations

** NSP = Non-pregnant serum

Table 11. Concentrations of sodium and potassium in 13-1/2 - 20-1/2 day old fetal serum.

Day	Number of Samples		Na^+ (mEq/L)*	K^+ (mEq/L)
13-1/2	8	Mean	144.0	17.2
		S.D.	±5.8	±1.7
		Range	140.0 - 151.0	15.3 - 18.8
14-1/2	8	Mean	146.7	14.4
		S.D.	±17.2	±1.2
		Range	123.0 - 164.0	13.1 - 15.8
15-1/2	10	Mean	160.0	18.86
		S.D.	±17.1	±1.59
		Range	144.0 - 178.0	17.8 - 20.7
16-1/2	10	Mean	144.5	25.55
		S.D.	±2.1	±0.49
		Range	143.0 - 146.0	25.5 - 25.9
17-1/2	10	Mean	155.0	20.0
		S.D.	±10.0	±2.7
		Range	139.0 - 169.0	17.4 - 24.8
18-1/2	8	Mean	143.7	12.94
		S.D.	±17.6	±1.56
		Range	126.0 - 163.0	12.6 - 17.1

Table 11 (continued)

Day	Number of Samples		Na^+ (mEq/L)	K^+ (mEq/L)
19-1/2	10	Mean	144.2	16.0
		S.D.	±4.3	±0.65
		Range	130.0 - 155.0	15.0 - 16.8
20-1/2	8	Mean	142.8	15.9
		S.D.	±8.16	±0.64
		Range	136.0 - 156.0	15.0 - 16.8

* = Concentrations measured in milliequivalents per liter (mEq/L)

S.D. = Standard Deviations

Table 12. Concentrations of sodium and potassium in 13-1/2 - 20-1/2 day fetal amniotic fluid.

Day	Number of Samples		Na^+ (mEq/L)*	K^+ (mEq/L)
13-1/2	10	Mean	126.0	6.30
		S.D.	±18.1	±1.70
		Range	107.0 - 156.1	3.9 - 8.7
14-1/2	10	Mean	123.4	4.6
		S.D.	±12.7	±1.7
		Range	109.0 - 146.5	3.0 - 8.7
15-1/2	10	Mean	145.9	4.5
		S.D.	±11.7	±0.7
		Range	137.7 - 158.6	3.1 - 5.4
16-1/2	10	Mean	134.1	3.9
		S.D.	±12.0	±0.4
		Range	112.4 - 155.0	3.4 - 4.4
17-1/2	10	Mean	133.7	4.3
		S.D.	±7.9	±1.4
		Range	120.0 - 140.6	3.5 - 4.5
18-1/2	10	Mean	122.1	4.4
		S.D.	±10.2	±0.9
		Range	110.5 - 136.0	4.3 - 5.8

Table 12 (continued)

Day	Number of Samples		Na^+ (mEq/L)*	K^+ (mEq/L)
19-1/2	10	Mean	141.4	4.5
		S.D.	±10.5	±0.3
		Range	122.4 - 155.9	4.3 - 5.0
20-1/2	10	Mean	135.2	4.6
		S.D.	±7.9	±1.7
		Range	120.0 - 140.6	3.0 - 8.7

* = Concentrations measured in milliequivalents per liter.

S.D. = Standard Deviations

concentration was on 13-1/2 days (6.30 mEq/L); the lowest was on 16-1/2 days (3.9 mEq/L). There were no significant changes in potassium concentrations from 14-1/2 - 20-1/2 days in studies on the amniotic fluid of rat fetuses.

Analyses of the Effects of DMSO on Protein Concentrations and Sodium and Potassium Levels in Maternal and Fetal Blood Serum and Amniotic Fluid

Total protein

When gravid rats were injected with 0.25 ml of 50% DMSO on day 14-1/2, there was a decrease in the total protein of maternal serum on day 15-1/2 to 5.5 g/100 ml when compared with non-treated gravid rat of 7.044 g/100 ml (Table 13). By day 19-1/2 the total protein was 5.6 g/100 ml, which is the same as that observed in the non-treated gravid females on day 19-1/2 of gestation.

In the 15-1/2-day fetuses of maternal rats that had been injected with DMSO, we observed that there was a slight decrease in the total protein of fetal serum (0.0530 g/100 ml) and a marked decrease in 19-1/2 day fetal serum (2.415 g/100 ml) when compared with that of untreated gravid female rats (Table 13). In the amniotic fluid of fetuses from treated rats there is also a slight decrease in the total protein when compared with the amniotic fluid of fetuses from untreated rats. On 15-1/2 days of fetal gestation the total protein was 0.530 g/100 ml and 0.950 g/100 ml on day 19-1/2 (Table 13). Figures 9 and 10 show a comparison between maternal serum, fetal serum, and amniotic fluid sodium and potassium concentrations from 13-1/2 - 20-1/2 days of gestation.

Table 13. Total protein changes in maternal serum, fetal serum, and amniotic fluid when treated with DMSO.

Day		Number of Samples	Mean	S.D.	Range
15-1/2	MS	10	5.5	±0.366	4.9 - 5.7
19-1/2	MS	10	5.80	±0.137	5.6 - 5.9
15-1/2	FS	4	0.53	±0.090	0.44 - 0.65
19-1/2	FS	8	1.88	±1.050	0.91 - 3.60
15-1/2	AF	10	0.0950	±0.043	0.42 - 0.17
19-1/2	AF	10	0.2142	±0.106	0.25 - 0.06

MS = Maternal serum

FS = Fetal serum

AF = Amniotic fluid

Fig. 9. Comparison between maternal serum, fetal serum, and amniotic fluid sodium concentrations for 13-1/2 - 20-1/2 days of gestation.

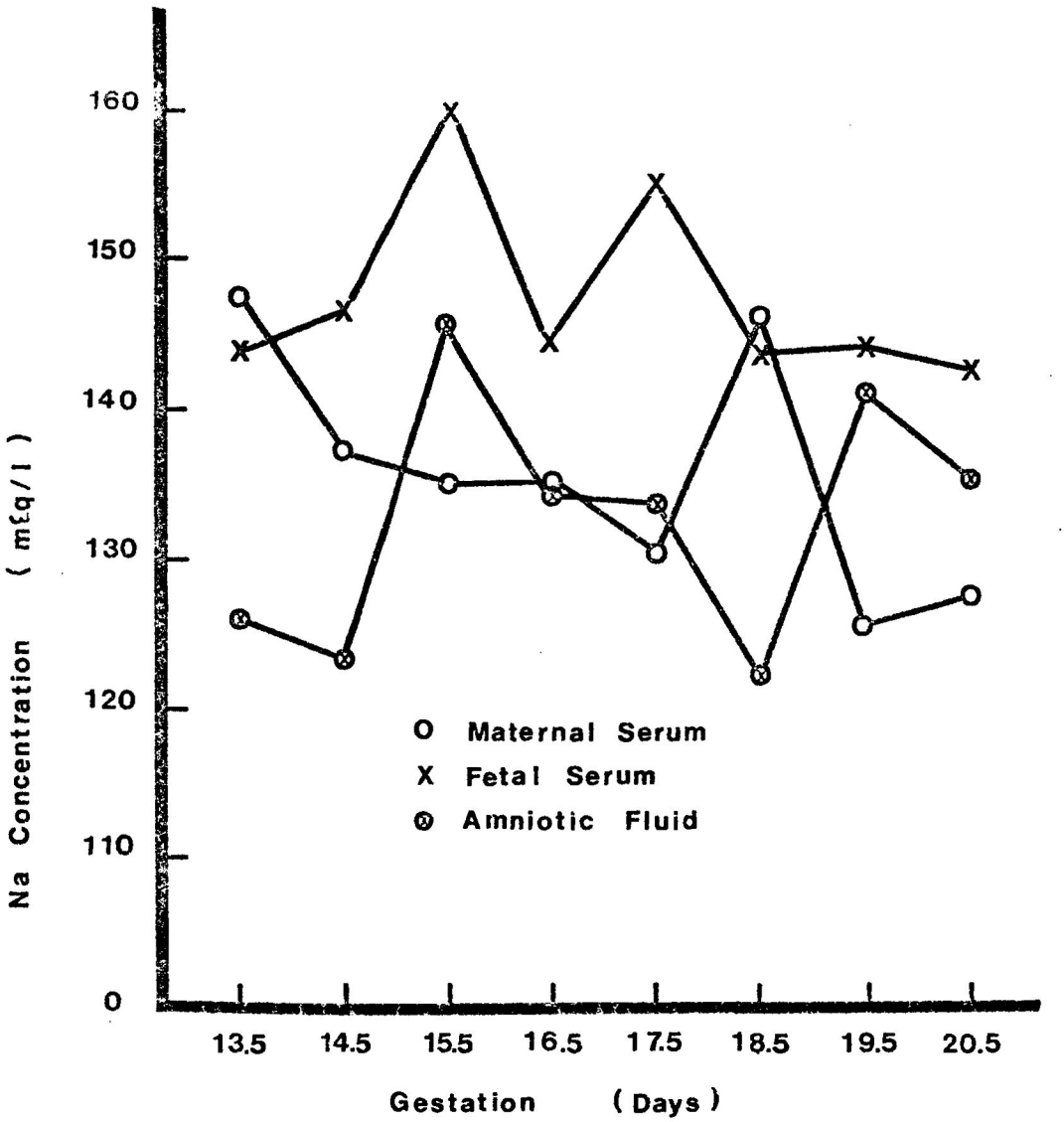
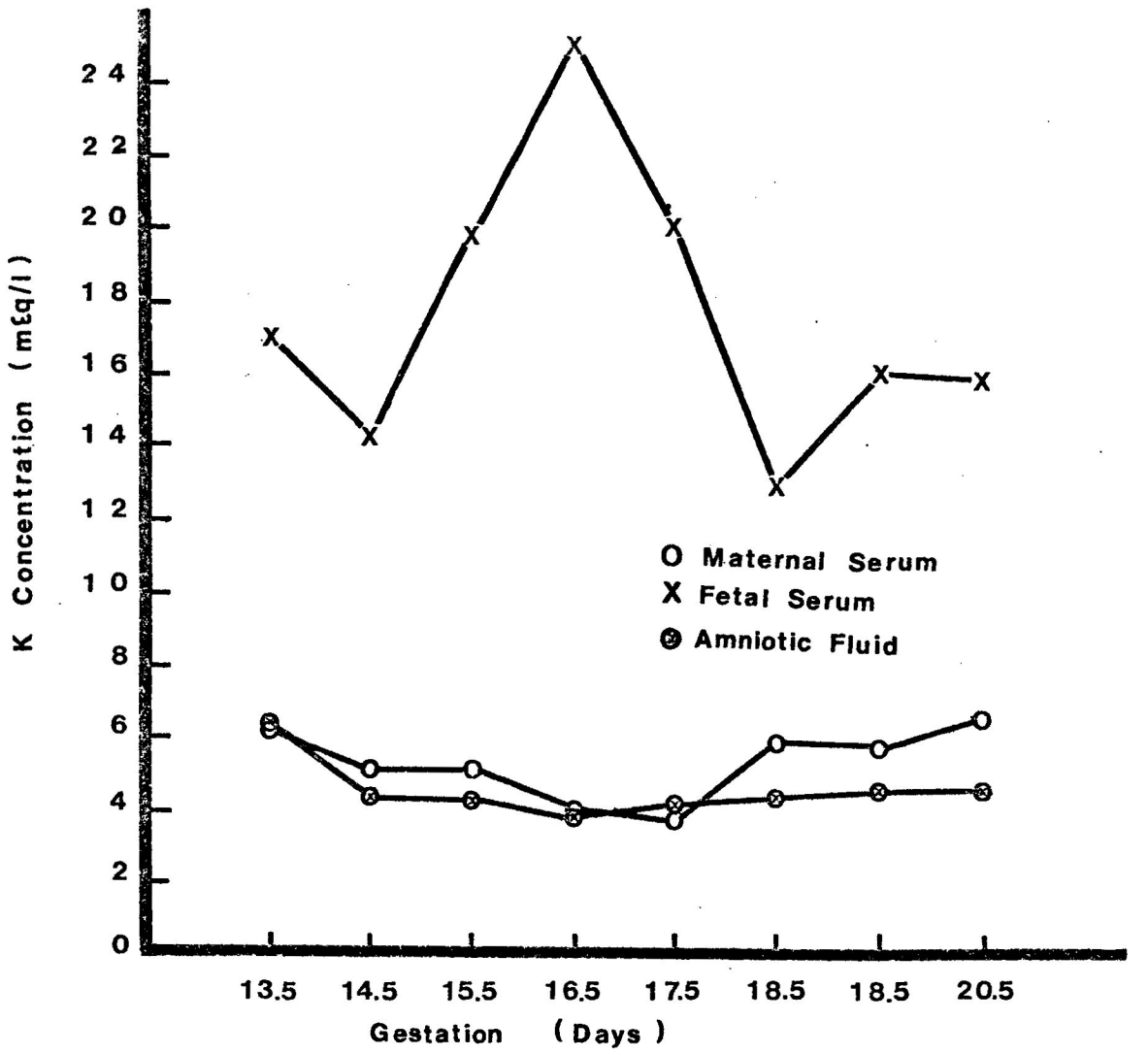

160
150
140
130
120
110
0
Na Concentration (mEq/l)
13.5
14.5
15.5
16.5
17.5
18.5
19.5
20.5
Gestation (Days)
O Maternal Serum
X Fetal Serum
⊗ Amniotic Fluid

Fig. 10. Graph showing a comparison between maternal serum, fetal serum, and amniotic fluid potassium levels for 13-1/2 - 20-1/2 days of gestation.


K Concentration (mEq/l)
24
22
20
18
16
14
12
10
8
6
4
2
0
O Maternal Serum
X Fetal Serum
⊗ Amniotic Fluid
13.5
14.5
15.5
16.5
17.5
18.5
18.5
20.5
Gestation (Days)

Gel-electrophoretic analyses

When gravid females were injected with DMSO a gradual increase in maternal serum protein fractions was observed, in particularly in the alpha, transferrin and gamma fraction on day 15-1/2 of gestation. On day 19-1/2 there was a marked increase in the albumin fraction of the maternal serum to an average of 36%. In contrast the concentrations of alpha and beta fractions were lower in the DMSO-treated gravid rats than in the untreated gravid ones (Table 14).

In DMSO-treated gravid female rats 60% of the protein in the 15-1/2-day fetal serum was albumin and in the amniotic fluid 67% was albumin (Table 15). The prealbumin was lower in the blood serum of untreated fetuses at 15-1/2 days (13.8%) than in the treated (20.2%), and about the same in the untreated 19-1/2 day fetuses (23.1%) when compared to the analyzed fractions treated for 19-1/2 day fetuses (24.4%). In the amniotic fluid the prealbumin was higher in the untreated animals.

Sodium and potassium analyses of fluids from DMSO-treated rats

When gravid rats were injected with DMSO on 14-1/2 days of gestation the mean sodium concentrations in the maternal blood serum were 135.4 mEq/L on 15-1/2 days and 126.7 mEq/L on 19-1/2 days of gestation. The mean potassium concentrations were 5.3 and 5.5 mEq/L for 15-1/2 and 19-1/2 days of gestation, respectively. These resulting data were very close to those observed in untreated gravid female blood serum (Table 16).

In blood serum of 15-1/2-day old fetuses of DMSO-treated maternal hosts, the mean sodium concentrations were 150.0 mEq/L and 128.0 mEq/L

Table 14. Electrophoretic distribution of proteins in maternal serum of DMSO-treated rats in relative percent.*

Day		Albumin	Alpha-1	Alpha-2	Alpha-2[1]	Transferrin	Beta	Gamma-1	Gamma-2
15-1/2	M.	31.8	5.1	7.2	12.9	6.8	17.0	14.6	4.5
	S.D.	±4.3	±0.5	±0.9	±1.6	±1.1	±3.2	±1.0	±0.2
	R.	28.5 - 36.7	4.6 - 5.5	6.4 - 8.3	10.9 - 14.0	5.4 - 7.5	14.2 - 20.7	13.8 - 15.8	4.0 - 4.5
19-1/2	M.	36.0	4.0	5.9	8.8	5.0	18.1	16.2	6.0
	S.D.	±1.2	±0.6	±2.3	±0.4	±0.2	±1.6	±0.2	±0.1
	R.	35.2 - 37.1	3.7 - 4.5	4.2 - 7.5	8.5 - 9.1	4.9 - 5.3	17.0 - 19.3	16.0 - 16.1	6.0 - 6.1

* Expressed in percentage

M. = Mean

S.D. = Standard Deviations

R. = Range

Table 15. Electrophoretic distribution of serum and amniotic fluid proteins from fetuses of DMSO-treated maternal rats in relative percent.*

Day											
15-1/2	FS	M.	20.2	40.4		16.5		16.8		6.1	
		S.D.	±5.2	±0.848		±0.707		±0.986		±0.56	
		R.	18.3 - 23.4	40.0	- 41.0	16.0	- 17.0	14.5	- 17.0	6.0	- 6.9
19-1/2	FS	M.	24.4	34.9		16.8		15.1		8.8	
		S.D.	±0.9	±3.7		±1.3		±3.9		±0.66	
		R.	23.0 - 25.5	29.6	- 38.1	11.6	- 19.8	15.9	- 18.7	7.0	- 9.7
15-1/2	AF	M.	15.0	52.7		10.0		14.2		8.1	
		S.D.	±2.0	±2.6		±2.1		±2.0		±1.3	
		R.	12.9 - 19.1	48.1	- 55.0	8.03	- 14.9	12.0	- 18.0	5.7	- 10.0
19-1/2	AF	M.	16.8	50.5		12.3		11.6		8.8	
		S.D.	±4.4	±5.2		±3.6		±1.7		±1.9	
		R.	10.5 - 20.5	42.6	- 53.5	7.5	- 15.6	9.6	- 12.9	6.3	- 11.0

*Expressed in relative percent. M = Mean S.D. = Standard Deviation R = Range
FS - Fetal serum AF = Amniotic fluid

Table 16. Concentrations of sodium and potassium in DMSO-treated maternal serum, fetal serum, and amniotic fluid for 15-1/2 and 19-1/2 days of gestation.*

	Samples				
15-1/2	MS	10	M.	135.4	5.3
			S.D.	±7.6	±0.8
			R.	120.0 - 147.0	4.0 - 6.5
19-1/2	MS	10	M.	126.7	5.5
			S.D.	±3.6	±0.7
			R.	115.0 - 144.0	5.2 - 6.4
15-1/2	FS	6	M.	150.0	18.5
			S.D.	±15.0	±3.7
			R.	131.0 - 176.0	12.0 - 23.8
19-1/2	FS	8	M.	128.0	15.9
			S.D.	±14.5	±3.9
			R.	109.0 - 148.0	9.0 - 20.0
15-1/2	AF	10	M.	133.4	3.7
			S.D.	±5.8	±0.9
			R.	120.0 - 139.9	3.1 - 4.2
19-1/2	AF	10	M.	126.0	4.7
			S.D.	±14.0	±0.4
			R.	109.0 - 146.0	3.8 - 4.7

*Concentrations measured in milliequivalents per liter.

MS = Maternal serum FS = Fetal serum AF = Amniotic fluid

in 19-1/2-day old fetuses. The potassium blood serum concentrations were 18.5 and 15.9 mEq/L for 15-1/2- and 19-1/2-day old fetuses, respectively. These resulting concentrations were higher than those observed in untreated animals in 15-1/2- and 19-1/2-day old fetuses (Table 16). In the amniotic fluid of fetuses of DMSO-treated maternal rats the mean sodium levels were 133.4 mEq/L on 15-1/2 days and 126.0 mEq/L on 19-1/2 days. The mean potassium levels were 5.3 mEq/L for 15-1/2 days and 4.2 mEq/L on 19-1/2 days of gestation in the amniotic fluid (Table 16).

CHAPTER V

DISCUSSION

Protein Analysis

Of all known chemical compounds proteins are the most complex and the most characteristic of living matter. Using the biuret method (Gornall et al., 1949), we have conducted a comparative study of the total protein of maternal serum, fetal serum and amniotic fluid, from 13-1/2 - 20-1/2 days of gestation in the Long-Evans rat. The study reveals that there is constant fluctuation in the concentration of proteins during these denoted periods of gestation.

In the maternal serum the concentration of the protein is not in a steady state. The protein concentration fluctuates from a high of 7.1 g/100 ml on 14-1/2 and 15-1/2 days of gestation to a low of about 5.8 g/100 ml on days 18-1/2 - 20-1/2 of gestation. This fluctuation in the total protein is probably due to the physiological changes that the gravid females experience during the gestational period. On a whole, the total protein concentration of maternal rat serum is higher than that of non-pregnant rat serum (6.1 g/100 ml). In maternal rat serum there is a higher concentration of globulins than albumin. This is in opposition to the work of Wirtschafter and Williams(1957) whose results show that there is a higher concentration of albumin than globulins in the maternal serum of rats. Our data show that serum contains from 31-32% albumin. In human beings, albumin constitutes about 45% of the total proteins at term (McKay et al., 1958; Viergivers et al., 1962).

Also, Moore et al. (1962) reported that albumin in the maternal serum decreased during pregnancy. In the Long-Evans rat, however, no significant decrease in the albumin is noted with the gel or cellulose acetate electrophoresis studies. The albumin fraction is in fact higher in the cellulose acetate electrophoretic studies. We think this is due to the fact that the cellulose acetate system did not give the well-defined separations shown in the gel electrophoretic bands. The alpha-1 and beta fractions are higher and the gamma fraction is lower in the cellulose acetate plate studies than in the gel electrophoretic studies. The alpha and beta globulins of maternal rat serum are greatly increased over those of non-pregnant rat serum. This is also the case in human beings (Moore et al., 1962).

The total protein for both amniotic fluid and fetal serum is much lower than that of maternal serum. However, total protein values are lowest in the amniotic fluid. It is markedly noticeable that the prealbumin and albumin fractions are conspicuously unstable and continuously fluctuate during the entire gestation period. These fractions represent from 55 to 65% of the total protein in the amniotic fluid. The same conditions prevail in the fetal serum where 51 to 60% of the total protein constituency is prealbumin and albumin. Wirtschafter and Williams (1957) reported that by 22 days of gestation in the Long-Evans rat, the albumin fraction represented more than 50% of the total protein. Our data support this contention. However, their data also indicate that unlike serum protein levels, amniotic fluid contains a higher concentration of globulin than albumin until the 21st day of gestation. The results obtained in our protein fractions studies are

considerably different. From 13-1/2 to 20-1/2 days of gestation the albumin fractions are always greater than the globulin fractions. Marsh et al. (1960) reported that from 18 to 22 days of gestation in the rat, the albumin fraction did not exceed 28.6% and that the alpha globulin fraction represented from 51 to 68.8% of the total protein.

Our data suggest that in amniotic fluid albumin is continuously being synthesized, although at different rates during the gestation period. The large amounts of prealbumin observed on 13-1/2, 15-1/2, 19-1/2, and 20-1/2 days of gestation probably represents increased turnover rates for albumin, possibly due to the many biochemical and physiological changes that occur in the fetuses and/or amnion itself during these periods. According to Kelsey (1971), albumin is important as a nutritional factor in tissue protein metabolism. Its most important function is stabilization and regulation of intravascular and extravascular fluid exchange under normal vascular conditions. Albumin acts as a vehicle for the transport of many cations, anions, and pigments. It also contributes to their excretion, solubilization, stabilization and diffusion into tissues. Furthermore, albumin is important in the stabilization and/or solubilization of the relatively insoluble globulins.

The highest mean alpha globulins that we obtained during our entire study of amniotic fluid were 21% (cellulose acetate electrophoresis) and 18.3% (gel electrophoresis). We also noted a trend of decreasing concentration in the alpha globulin fraction. Marsh et al. (1960) found the lowest concentration for the alpha globulin fraction to be 51% at 20 days of gestation. They also noted a decreasing trend

in the alpha globulin fraction as gestation advanced (18-22 days). These workers surmised that the high percent of alpha globulin that they obtained in their studies probably could be attributed to the increased viscosity of rat amniotic fluid from 18-22 days of gestation. In our sequential studies we, too, observed empirically that as gestational days increased, so did the relative viscosity of the amniotic fluid. This statement is made on the basis of ease or difficulty involved in amniotic fluid extraction. But from our data we cannot postulate that albumin or alpha globulin are responsible for this increased viscosity. One main detractant to this postulation is that on 13-1/2 days of gestation, the albumin mean fraction is 61.8% and the alpha fraction is 18.5% of the total protein. Subsequently, on 18-1/2 days of gestation we could readily observe an increase in the relative viscosity of the amniotic fluid when the total albumin fraction is 64% and alpha globulin fraction is 6.4% of the total protein.

The beta fraction in the amniotic fluid increased from 13-1/2 - 20-1/2 days of gestation except for a slight decrease on 17-1/2 and 18-1/2 days of gestation. However, even on 17-1/2 and 18-1/2 days of gestation it was still higher than the beta fraction on 13-1/2 days of gestation. There is a significant change in the beta fraction if you compare 13-1/2 days with that of 19-1/2 and 20-1/2 days of gestation. The graphic presentation of Wirtschafter and Williams (1957) showed very little change in the absolute amounts of beta and gamma globulins from 18 to 22 days of gestation. Marsh et al. (1964) also observed that there was no decline in the percent of the two fractions. Our

results from 18-1/2 - 20-1/2 days of gestation indicate that for the beta globulin there is a gradual increase in its percentage. As for the gamma globulin there is a slight decline in its concentration. Our results show that the globulin fractions fluctuated during the gestation period but not markedly.

In gravid human beings, the beta and gamma fractions have the same concentration (11%) and the alpha fractions were 11.6% of the total protein at term, according to McKay et al. (1955). Heron (1966) reported that at 36 weeks of gestation in human beings the beta and gamma fractions approximate each other in percent, but are not necessarily the same. The percentages of the alpha fractions reported by McKay et al (1958), Heron (1956), and Wirtschafter and Williams (1957) are markedly less than reported by Marsh et al. (1964).

The total protein in fetal serum exceeds that of the amniotic fluid and it increases as gestation increases. On the whole the albumin fractions in the fetal serum are less than in amniotic fluid. Prealbumin in fetal serum is lower than that of amniotic fluid from 13-1/2 to 16-1/2 days of gestation. This suggests that the turnover of albumin is less in the fetal serum from 13-1/2 - 16-1/2 days of gestation when compared with the turnover rate of albumin in the amniotic fluid. From 17-1/2 - 20-1/2 days there are marked increases in the prealbumin which could indicate that the turnover rate of albumin increases as the fetuses mature. This implies that as the fetuses progress toward term there is an increase in the *in situ* physiological functions that the albumin controls or plays a regulatory role in maintaining. Among the roles postulated could be stabilization of

blood volume and regulation of intravascular and extravascular fluid exchanges; likewise, the transport of cations, anions and pigments may be included.

The alpha fraction is lower in the fetal serum than in the maternal serum. It approximates that found in normal serum. The gamma fraction in the fetal serum was also found to be much lower than that observed in non-pregnant and maternal rat serum. Longworth et al. (1945) postulated that human fetal serum is much closer in composition to non-pregnant female serum than maternal serum. The most noticeable discrepancy was in the increased proportion of gamma globulin in the fetus, when compared with non-pregnant female serum. Our results suggest that rat fetal serum proteins are closer in concentrations to the amniotic fluid than to those of maternal and non-pregnant rat serum.

Sodium and Potassium Concentrations

In the maternal blood serum of the Long-Evans rat there is a decreasing trend in the sodium concentration with gestational age of in utero fetuses. The maternal serum sodium concentration is lower than that of the non-pregnant rat serum. Sodium concentration in the amniotic fluid from 13-1/2 - 14-1/2 days of gestation starts initially at a decreased level and then at 15-1/2 days abruptly increases, followed on days 16-1/2 to 19-1/2 by a conspicuous decrease and increasing again on day 19-1/2. Noticeably at 19-1/2 and 20-1/2 days the concentration of sodium is still not as high as it was on 15-1/2 days of gestation.

The observed decrease in the concentration of sodium in the

fetal serum is very similar to that in the amniotic fluid. There is a sharp increase at 15-1/2 days of gestation; then there is a decrease until 20-1/2 days. However, the concentration in the fetal serum exceeds that of amniotic fluid during the same gestational period. This low sodium concentration in the amniotic fluid may be caused by the secretion of urine into the amniotic fluid by the fetus. According to Lind (1973), the human fetal kidney starts functioning as early as 12 weeks of gestation or at the beginning of the second trimester. In human maternal serum osmolality and sodium levels do not change with increasing gestation. Also, there is a decreasing trend in the osmolality and sodium concentrations in the amniotic fluid from 30-38 weeks of pregnancy (Lind and Cheyne, 1971; Battaglia et al., 1959).

The potassium levels of maternal rat serum and amniotic fluid differ only slightly with advanced pregnancy, amniotic fluid had the lowest concentration. This is in agreement with the work of Lind and Cheyne (1971) in human beings. Our data show that the potassium levels in the fetal serum greatly exceed those of maternal serum and amniotic fluid.

The biochemical composition of amniotic fluid changes progressively as pregnancy advances. It could probably be that increasing stratification of fetal skin impedes and eventually prevents diffusion. From then on the changing biochemical characteristics could reflect the maturation of the fetal renal function and the activities of cells that are found in the amniotic fluid. The biochemical and physiological changes that occur in the fetus as gestation advances probably also have some direct effect on the changes that occur in the amniotic fluid.

Dimethylsulfoxide

Serum samples from maternal rat injected on day 14-1/2 of gestation with DMSO and analyzed on 15-1/2 days of gestation reveal a drop in the total protein concentration to 5.5 g/100 ml on the 15-1/2 days of gestation, which is much lower than the total protein of untreated rats on 15-1/2 days of gestation (7.04 g/100 ml). Subsequently, at 19-1/2 days of gestation the total protein concentration is the same for the treated and untreated maternal rat serum. There were no marked changes in the protein concentration of the amniotic fluid on these days. The fact that DMSO did not cause a change in the total protein on 19-1/2 days of gestation is probably due to the excretory activities occurring in the rat's system. After 24 hrs dimethyl sulfone is excreted in the urine and dimethyl sulfide is eliminated in the expired air (Wong et al., 1971). Both of these substances are end products of DMSO breakdown. This physiological activity allows the maternal serum sample time for readjustment. On 15-1/2 days of gestation, 24 hrs after the injection with DMSO, many of the biochemical and physiological changes that occur upon the administration of DMSO were observed because the limited time period did not allow for stabilization, thus the observed increase in the total protein. It was suggested by Williams et al. (1965) that because of its comparatively small size DMSO was able to penetrate regions of the protein subunits interfaces more readily than other bulkier solvents. Dimethylsulfoxide also appears to be extremely effective in altering the configuration of proteins and this change is apparently reversible after removal of DMSO (Rammler, 1966).

The amniotic fluid protein fractions from fetuses of DMSO-treated maternal rats (15-1/2 days) show marked increases in albumin fractions and marked decrease in the alpha and gamma fractions when compared to the untreated amniotic fluid. There is also an increase in the albumin fractions and a decrease in the alpha and gamma fractions for 19-1/2 days but they are not as great as those for 15-1/2 days of gestation. Thus, our data show that DMSO has a direct effect on the protein levels in the amniotic fluid. This could occur by the absorption of DMSO through the amniotic sac or by way of fetal secretions. In man, after the administration of DMSO, relative amounts of DMS and $DMSO_2$ can be found in the plasma and urine, respectively (Wong et al., 1971).

In fetal serum we noted a marked increase in the prealbumin fraction on 15-1/2 days of gestation, and the albumin fraction is almost the same as in untreated rats. The other fractions are very similar in concentration to the normal. Fetal serum protein fraction for 19-1/2 day fetuses of DMSO-treated maternal rats are very close to the untreated. Here again, as in the maternal serum, it appears that DMSO is affecting the levels of proteins more so 24 hrs after injection in the 15-1/2-day fetuses than in the 19-1/2-day fetuses, which is 96 hrs after injection of DMSO.

There are no significant changes in the sodium and potassium levels in the DMSO-treated gravid females at 15-1/2 and 19-1/2 days of gestation. However, in the amniotic fluid and fetal serum of DMSO-treated rats for 15-1/2 and 19-1/2 days of gestation, there are marked decreases in the sodium concentrations when compared to the untreated rats (Table 16). The potassium levels are very close to

those of the untreated rat. It appears that DMSO is causing electrolyte disturbances in the fetal serum and amniotic fluid. These changes in the fetal serum electrolytes could have a direct effect on the developing fetus. Browne (1968, 1970) showed that these fluid and electrolyte disturbances in embryonic and extra-embryonic compartments could lead to induced anomalies in the chick embryo.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The results of our overall investigation into the role of macromolecules in the ontogeny of biochemical characterization and maintenance of amniotic fluid show that:

1. During pregnancy the proteins of maternal serum, fetal serum, and amniotic fluid are constantly changing. The total protein concentration of non-pregnant serum is lower than that of pregnant serum and the total protein concentration of amniotic fluid and fetal serum increases as pregnancy advances in the rat.

2. The results of our investigations did not support the idea that the concentration of alpha globulin causes an increase in the viscosity of the amniotic fluid with advancing gestation. This postulation is strikingly unfounded on the basis of no such marked increases in the alpha globulins previously reported.

3. The amniotic fluid is more similar to the protein composition of fetal serum than to the maternal serum from 13-1/2 - 20-1/2 days of gestation in the rat.

4. The large amounts of prealbumin observed of amniotic fluid and fetal serum suggest that the turnover rate in albumin in the amniotic fluid and fetal serum is rapid, orderly, and possibly regulated.

5. Amniotic fluid has a larger concentration of albumin than fetal serum as gestation advances. In both amniotic fluid and fetal serum the concentration of albumin is greater than that of maternal and non-pregnant rat serum.

6. The sodium and potassium concentration of maternal serum and amniotic fluid are very close during gestation. The sodium levels gradually decline with advancing gestation.

7. In amniotic fluid and fetal serum there is a sharp increase in the sodium concentration on 15-1/2 days of gestation, after which there is a gradual decline. We suggest that this is due to the onset of functions in the fetal kidney.

8. The potassium levels in fetal serum greatly exceed those of maternal serum and amniotic fluid and this is probably due to the dynamic biochemical and physiological changes that are occurring in the developing fetus.

9. Twenty-four hours after the injection of DMSO into gravid female rats, there is a significant decrease in the total protein of maternal serum. The induction of DMSO into the rat's system can cause the concentration of protein fractions to change. DMSO also causes a decrease in sodium levels in fetal serum and amniotic fluid.

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